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Effects of the Prostaglandin Synthesis Inhibitor, Indomethacin on Estrogen- and Estrogen plus Progesterone-Induced Sexual Receptivity in Ovariectomized Rats[1]

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HALL, N. R. AND W. G. LUTTGE. *Effects of the prostaglandin synthesis inhibitor, indomethacin on estrogen- and estrogen plus progesterone-induced sexual receptivity in ovariectomized rats.* PHARMAC. BIOCHEM. BEHAV. 8(5) 597–602, 1978. – Implants of crystalline PGE_2 in the basal preoptic-anterior hypothalamic areas stimulates high levels of sexual receptivity in ovariectomized, estrogen-primed rats. Indomethacin, which blocks the synthesis of PGE_2, failed to inhibit either estrogen- or estrogen plus progesterone-induced receptivity. Neither intracerebral nor subcutaneous administration of indomethacin diminished the display of steroid induced reproductive behavior without also causing a depression in open-field activity, and in some cases, causing gastrointestinal problems and even death. These results suggest that prostaglandin synthesis is not a required step in the mechanism by which estrogen and progesterone exert their behavioral effects. The possibility that PGE_2 and LH–RH synthesis and/or release might contribute to a collateral mechanism for the induction of sexual receptivity was discussed.

Prostaglandin E_2 Indomethacin Sexual receptivity LH–RH Open-field activity Hypothalamus Ovarian hormones

IN EARLIER work we have demonstrated that intraventricular [10] and intracerebral [8,9] implants of crystalline prostaglandin E_2 (PGE_2) can facilitate the induction of sexual receptivity in estrogen-primed ovariectomized rats. Regional analysis indicated that PGE_2 stimulated the highest levels of receptivity when implanted in the basal preoptic and anterior hypothalamic areas (POA–AH) [8]. Luteinizing hormone releasing hormone (LH–RH), which is released by administration of PGE_2 [1, 4, 11, 27], also stimulates receptivity following both systemic and POA–AH administration in estrogen-primed ovariectomized rats [6, 19–21, 28, 34]. Since PGE_2 implants in the basal POA–AH can release LH [26], we have speculated that sexual receptivity in female rats may be mediated via an estrogen + progesterone → PGE_2 → LH–RH → receptivity mechanism localized in these same brain regions [8,9]. Although the POA–AH brain areas have been implicated by a variety of techniques including estrogen and antiestrogen implantation [3, 14, 15, 17], radiolabeled estrogen localization [18, 29, 32], electolytic lesions [22,23] and electrical stimulation [22,24] as being involved in estrogen- and estrogen plus progesterone-induced sexual receptivity, our previous studies have not provided any firm evidence that these hormones must exert their behavioral effects via a molecular mechanism involving PGE_2.

In order to determine whether or not prostaglandin synthesis is necessary for the display of hormone-induced receptivity, female rats in the present series of experiments were administered indomethacin via either SC injection or intracerebral implantation. Indomethacin has been shown to block the action of prostaglandin synthetase which converts arachidonic acid to both PGE_2 and $PGF_{2\alpha}$ [5, 13, 36]. It was hypothesized that if prostaglandin synthesis is necessary for the display of sexual receptivity, then the administration of this drug during estrogen and pro-

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gesterone priming should block the display of sexual behavior.

METHOD

For each study adult female CD rats (275–325 g) were purchased from Charles Rivers Laboratories (Wilmington, MA) and housed individually with food and water available at all times. The animal colony lights were left on from 2100 to 0900 hr. Based on this time clock all injections were given between 0900 and 1200 hr and all behavioral tests were conducted between 1200 and 1600 hr. All females were ovariectomized under ether anesthesia.

Estradiol benzoate (1,3,5(10)-estratrien-3,17β-diol 3-benzoate) (E_2 B) and progesterone (4-pregnen-3-20-dione) were purchased from Steraloids, Inc. (Wilton, NH). Both hormones were prepared for SC injections by first dissolving and then diluting them in peanut oil to various concentrations so that all injections were delivered in volumes of 0.1 cc. The prostaglandin E_2 used in this study was generously provided by Dr. John E. Pike of the Upjohn Co. (Kalamazoo, MI), while the Indomethacin was obtained from Merck Sharp and Dohme (West Point, PA).

For sexual behavioral tests, each female was placed in a clear Plexiglas testing arena with a sexually vigorous stud male. Males were permitted to mount the female 10 times and the lordosis quotient (L/M × 100) was computed for each female. Open-field activity tests were conducted immediately following the receptivity test. Further details of both the sex and activity tests are published elsewhere [8,9]. Rectal body temperature for each animal was recorded using a Yellow Springs Instruments Model 43 single channel telethermometer.

Intracerebral PGE_2 and indomethacin were delivered in crystalline form using 28 g cannulas inserted through stereotaxically implanted 22 g guide cannulas [8,9]. Weighing these cannulas before and after filling on a Cahn G2 electrobalance indicated a differential weight ($\bar{X}$ ± SEM) of 4 ± 1 μg for PGE_2 and 16 ± 1 μg for indomethacin. Empty cannulas were used in control groups. At the conclusion of the experiment all females were anesthetized with ether, perfused with 10% formal-saline and the brains serially sectioned in order to confirm the implantation sites.

All data were analyzed by *a priori* two-tailed *t* tests or analysis of variance F tests. *A posteriori* comparisons were performed according to the Newman-Keuls procedure [35].

Experiment 1: Effects of Systemic Administration of Indomethacin on Estrogen plus Progesterone–Induced Receptivity

Procedure. Beginning one week after ovariectomy 32 CD female rats were started on a series of three weekly baseline sexual behavior tests. For each test females received SC injections of 1–4μg E_2 B at 51 hr and 500 μg progesterone at 3 hr, prior to testing. Threshold E_2 B doses were determined for each individual in order to stimulate receptivity using a minimum amount of hormone. Twenty-six females having displayed lordosis quotients of 50% or higher on the baseline tests were matched and assigned to one of four groups. One week later, Group 1 females (N = 8) received SC injections of 5 mg indomethacin at 0, 24 and 48 hr after the standard E_2 B injections for a total of 15 mg prior to testing, while Group 2 females (N = 8) received 0.1 cc SC injections of the 0.1 M sodium phosphate-buffered saline vehicle. Group 3 females (N = 5) received SC injections of 2.5 mg indomethacin at 24 and 48 hr after the standard E_2 B injections for a total of 5 mg prior to testing, while the Group 4 females (N = 5) received 0.05 cc SC injections of the indomethacin vehicle. Progesterone injections were given to all females as in the baseline tests. Rectal body temperatures were recorded before and after the behavioral tests. One week later, females in Group 3 were again treated with indomethacin, this time receiving SC injections of 2.5 mg at 0, 24, and 48 hr after the E_2 B injections for a total of 7.5 mg indomethacin prior to testing, while the females in Group 4 received the control vehicle injections. Both groups received progesterone injections and rectal body temperature recordings as in previous weeks.

Experiment 2: Effects of Intracerebral Administration of Indomethacin on Estrogen Plus Progesterone- and on Estrogen Alone–Induced Sexual Receptivity

Procedure. One week after ovariectomy 14 CD female rats were stereotaxically implanted with bilateral double walled cannulas over the basal-preoptic and anterior hypothalamic areas (POA–AH). Beginning one week later, females were started on a series of weekly baseline tests in order to establish a threshold dose of E_2 B for each female. The threshold was considered as the minimum dose of E_2 B which when followed by progesterone would elicit receptivity. Individual threshold doses were found to range between 2 to 8 μg E_2 B for this group. Prior to any tests with indomethacin all basal POA–AH implant sites were first tested with PGE_2. For these tests females were primed with threshold doses of E_2 B (SC injections) followed 48 hr later by unilateral implantation PGE_2. Of the 10 animals that had L/M scores of 50% or higher on the PGE_2 test, only six were used in the indomethacin tests due to clogged cannulus and loosened head pieces in the remaining four rats. On the following week threshold doses of E_2 B were again given and females were tested for receptivity 51 hr later. On the day of the tests, the prostaglandin-responsive rats received bilateral basal POA–AH implants of either indomethacin (total dose = 32 μg) (N = 3) or empty cannulas (N = 3) 2 hr prior to an SC injection of 500 μg progesterone and 5 hr prior to testing. One week later the implantation procedures were reversed so that all six rats were eventually tested with and without intracerebral indomethacin treatment.

A separate group of 15 ovariectomized female rats was stereotaxically implanted with bilateral double walled cannulas over the basal POA–AH area. Beginning approximately one week after surgery, all of the animals received daily SC injections of 2 μg E_2 B. Weekly tests for receptivity were conducted for the next four weeks. At the end of that time, five animals that met the *a priori* criteria of exhibiting L/M scores of 50 or higher received bilateral POA–AH implants of indomethacin and three hours later they were tested for sexual receptivity. The indomethacin implants were removed shortly after the behavioral test. This procedure of implantation and testing was repeated daily for three days. During this time, the animals continued to receive daily injections of 2 μg E_2 B.

RESULTS

Experiment 1

The lordosis quotient of Group 1 animals receiving

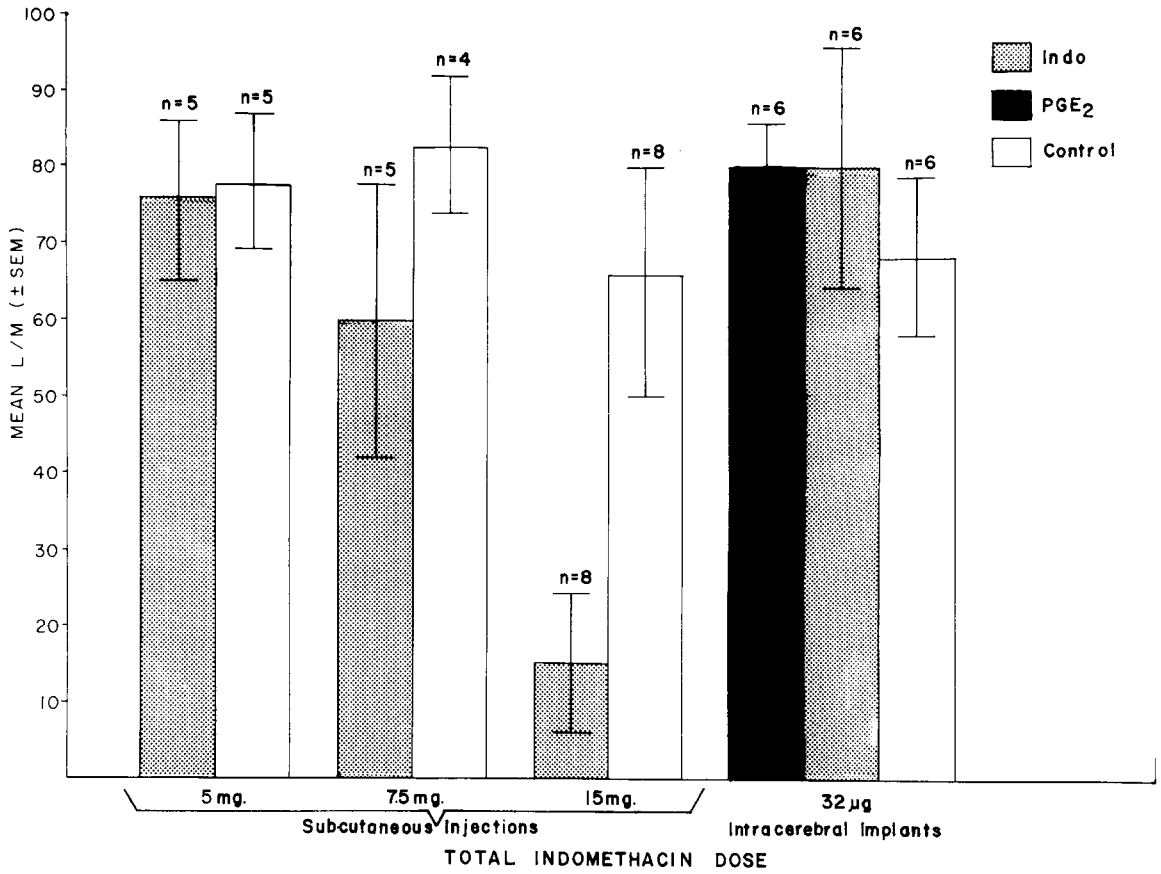

FIG. 1. Effects of indomethacin administration on sexual receptivity ($\bar{X}$ ± SEM lordosis quotients) in ovariectomized female rats. Strippled bars represent scores from estrogen plus progesterone-primed females receiving 5, 7.5 or 15 mg of indomethacin injected SC or 32 µg of crystalline indomethacin implanted directly into basal POA–AH brain areas. Open bars represent scores following control injections of vehicle or the implantation of empty cannulas. The solid bar represents the scores of estrogen-primed females receiving a basal POA–AH implant of 4 µg PGE_2.

15 mg of systemic indomethacin was reduced 75% when compared with the Group 2 control rats (Fig. 1) ($p<0.05$). Although these animals were nonreceptive following indomethacin, the open-field activity tests indicated that they were also extremely lethargic. Compared with controls, indomethacin treated rats had 70% fewer line crossings during the 5 min tests (Fig. 2) ($p<0.05$). Indomethacin treated animals also had a pretest (36.6 ± 0.2°C) and post-test (37.3 ± 0.2°C) mean body temperature that was significantly lower than the pretest (38.0 ± 0.2°C) and post-test (39.3 ± 0.2°C) values for the vehicle control group ($p<0.01$).

The reduced L/M and activity scores in the Group 1 females were most likely the result of poor health following indomethacin treatment. After three days of treatment, all of the animals had diarrhea and within four days after the final injection of the drug, seven of the eight treated rats were dead. Although body weights were not recorded prior to the administration of the indomethacin, on the day of the tests the mean weight of the treated animals (296 g) was significantly lower than the mean weight of the control group (331 g) ($p<0.01$).

Both the 5 mg and the 7.5 mg total dose indomethacin treatments given to the Group 3 females resulted in slight, but nonsignificant reductions in the lordotic behavior and in the open-field activity of the estrogen plus progesterone treated rats (Figs. 1 and 2). Although the health of the animals appeared normal following the 5 mg dose of indomethacin, two of the five animals that received the 7.5 mg dose died one week after the final injection. At the time of both tests all of the animals appeared to be in good health, but it is possible that the slightly reduced L/M and activity scores during the 7.5 mg test were due to a health problem that had not yet manifested itself through the animals appearance and behavior. Neither dose of indomethacin had any effect upon rectal body temperature in the Group 3 females.

Experiment 2

Unilateral implantation of PGE_2 into the basal POA–AH areas resulted in high levels of receptivity in ovariectomized females primed with threshold doses of estrogen (Fig. 1). When indomethacin was implanted bilaterally into these same areas the lordosis quotients for the females primed with threshold doses of estrogen and 500 µg progesterone remained at the high levels observed with the PGE_2 implants. Although the mean L/M score was slightly lower following tests with empty cannulas, this reduction failed to reach statistical significance.

Intracerebral indomethacin treatment resulted in a sig-

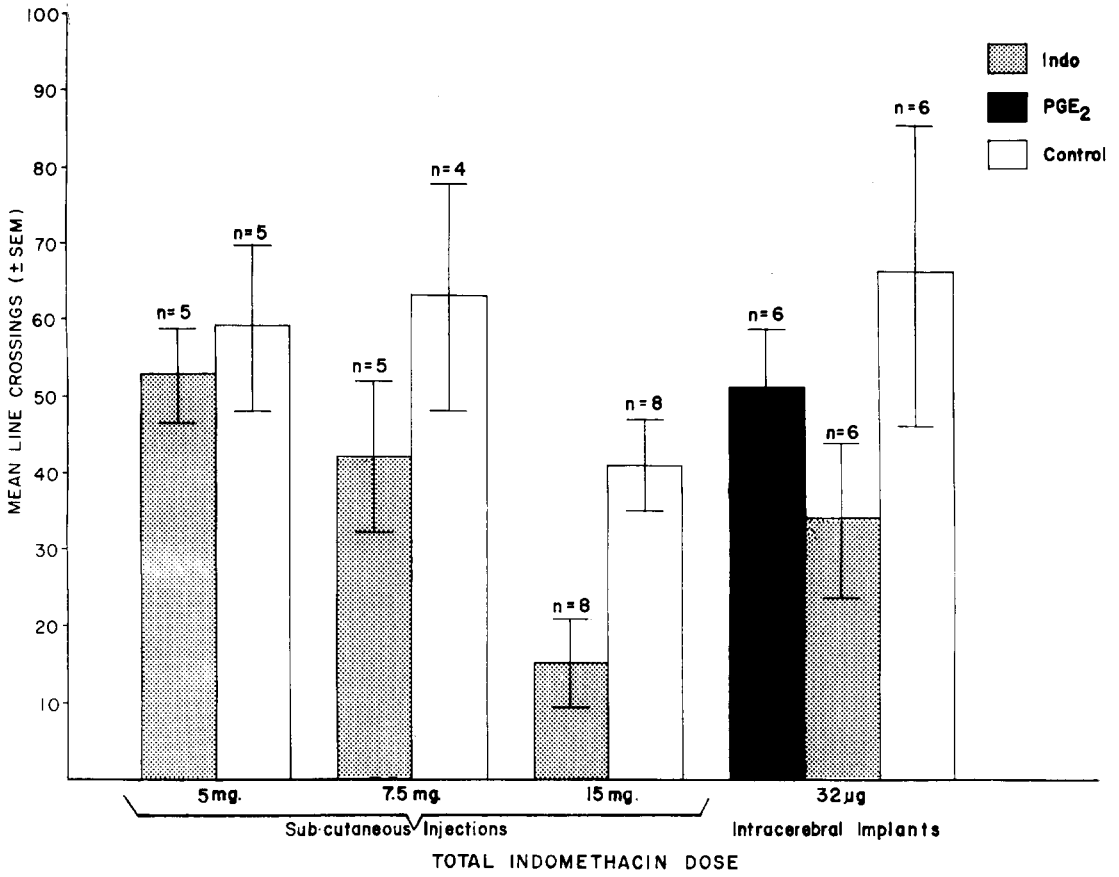

FIG. 2. Effects of indomethacin administration on open-field activity ($\bar{X}$ ± SEM) in ovariectomized female rats. Other details are explained in the legend of Fig. 1.

nificant decrease in the mean number of line crossings compared with the empty cannula condition (Fig. 2) ($p<0.05$). However, this decrease in activity was not correlated with a decrease in sexual responsiveness as it was in the experiments using systemic injections of indomethacin. Intracerebral PGE_2 treatment had no effect on open-field activity scores. No significant effects of indomethacin on rectal body temperature were observed.

The mean lordosis quotient for the final pretest of the ovariectomized females receiving chronic estrogen treatment was 90 ± 4%. Bilateral implants of indomethacin did not significantly reduce these scores. Three hr after the first implants of indomethacin, the mean lordosis quotient was 68 ± 18%. During this test, one rat had a score of 0% while the remaining four had L/M scores ranging from 70 to 100%. The indomethacin given 24 hr later also had no significant effect on the group L/M scores which averaged 92 ± 5%. After the third implant, the mean score for the group was 62 ± 17%, but as in the first test one animal had a score of 0% although it was not the same rat that exhibited a 0% score on the first test. That animal had L/M scores of 100% and 80% on the second and third tests.

DISCUSSION

Endogenous prostaglandin synthesis and release in the brain, coupled with LH–RH actions, has been hypothesized as part of the mechanism whereby the sex steroids estrogen and progesterone exert their behavioral effect [8–10]. The results of the present series of experiments do not entirely support this hypothesis. Although the systemic administration of 15 mg indomethacin did block estrogen plus progesterone-induced receptivity, open-field activity levels were also inhibited. These results suggest that the inhibitory effects of indomethacin on behavior were generalized and were the consequence of poor health. Lower doses of indomethacin failed to reduce significantly either receptivity or open-field activity. The 7.5 mg total dose of indomethacin did result in a nonsignificant decrease in the average L/M scores, but the activity scores were also reduced and one week later, two of the animals had died. Consequently, it was impossible to conclude whether the inhibitory effects of indomethacin on receptivity were due to the inhibition of prostaglandin synthesis in brain areas mediating reproductive behavior, to the indirect effects of poor health or to a combination of both consequences.

Most of the observed health problems appeared to be due to the well documented effects of indomethacin on the gastro-intestinal system. A rarer complication is toxic hepatitis [5,31] which, if present, could alter the metabolism of estrogen and progesterone. In order to minimize these adverse effects of indomethacin, the drug was implanted directly into the POA–AH brain regions. Pretests established that PGE_2 was capable of eliciting receptivity at these sites, but the result with the indomethacin implants revealed that the drug failed to inhibit receptivity even when administered in this manner.

Since sexual receptivity can be elicited with either chronic E_2 B alone or E_2 B plus progesterone, the possibility was considered that prostaglandin synthesis might be necessary for the display of E_2 B alone-induced receptivity, but not for E_2 B plus progesterone-induced receptivity. But intracerebral implants of indomethacin also failed to inhibit L/M scores in chronic E_2 B alone treated animals.

One interpretation of the data is that the indomethacin produced an insufficient reduction of prostaglandin synthesis so that LH–RH was still released in adequate amounts to induce sexual receptivity. LH–RH was not measured in the present experiment, but Ojeda *et al.* [25] have shown that systemic and intracerebral indomethacin administration can dramatically inhibit LH release. They also found that IV injections of LH–RH were capable of overcoming the indomethacin blocked LH release, suggesting that indomethacin is capable of acting at the level of the hypothalamus following intracerebral administration. Although not all of the conditions of the Ojeda *et al.* [25] study were replicated in the present study, their results, together with those of the present series of experiments, suggest that while PGE_2 can, under certain circumstances, induce sexual receptivity, PGE_2 synthesis and/or release may not be a necessary mechanism through which chronic E_2 B or E_2 B plus progesterone exert their behavioral effects. However, our data do not preclude the possibility that two independent mechanisms for the induction of sexual receptivity exist: one requiring and one not requiring a prostaglandin → LH–RH mechanism. The former possibility is supported by the finding that vaginal-cervical stimulation can elicit lordosis responses in the absence of both endogenous and exogenous ovarian hormones and that the same form of stimulation can elicit LH–RH release [2, 12, 30, 33]. While sexual receptivity in gonadally intact female rats may be primarily elicited through the direct brain actions of endogenous estrogen and progesterone, the brain actions of prostaglandins and LH–RH (released in response to either ovarian hormones or vaginal-cervical stimulation) may also contribute by potentiating lordotic behavior in marginally receptive females and/or by potentiating lordotic behavior following repeated mating tests [6,7].

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